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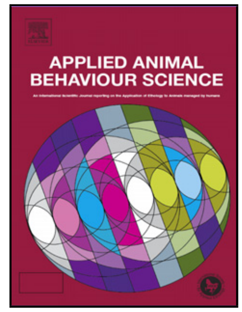

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The effects of enrichment novelty versus complexity in cages of group-housed rats (*Rattus norvegicus*)

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Highlights

- The study examined whether effects of environmental enrichment are due to novelty or complexity.
- Behavioural, performance and pathological indicators of welfare were used.
- The effects of enrichment are mainly due to complexity of the cages by multiple items.
- The type of environmental enrichment protocol affects welfare of laboratory rats.

Abstract

Although experimental work on environmental enrichment has answered many important questions, it is not yet known whether beneficial effects of enrichment are more strongly influenced by regular provision of novel objects, or by the diversity of objects present at any one time. In a five-replicate study, 80 newly weaned male Wistar rats were housed in groups of four in: a ‘novelty’ condition (NC) in which five copies of the same object were provided in the home cage during the first week of the study followed by five copies of a different, novel, object over each of the next 4 weeks; a ‘complexity’ condition (CC) in which one example of each of the five objects used in NC was always present in the cage across the 5-week study period. Behaviour was collected in two 2-hrs sessions every week, once during the light phase of the light/dark cycle and once during the dark phase. Body weight and weight gain were measured over the five weeks and organ weights were recorded post-mortem. Rats in the CC displayed elevated levels of measures suggestive of improved welfare such as sleep, enrichment-directed behaviour, enrichment contact, weight gain and relative thymus weight, and decreased levels of indicators denoting poor welfare including aggression, awake-non active and audible vocalizations. It appears that CC may have the potential to increase the frequency and diversity of behaviours, to express different types of behaviour when desired, and to augment the ability of the animals to exert some control over their environment, findings that may improve their welfare. Provision of novelty per se in the absence of diversity of objects at any one time seems to be less beneficial. Order (replicate) effects in the NC group indicated that responses of the animals towards the enrichment regimen appeared to depend on the type of object supplied, thus drawing attention to the importance of object characteristics when designing enrichment protocols for laboratory animals.

Key words: Laboratory Rats, Enrichment, Complexity, Novelty, Welfare.

1. Introduction

Laboratory rodents are typically housed in small barren cages that lack key features of their natural environment. This housing environment has been shown to impose constraints on the animals' behaviour, physiology and development, to compromise their welfare and to be stressful (e.g. Patterson-Kane et al., 1999; van Praag et al., 2000; Würbel, 2001; Van Loo et al., 2002; Johnson et al., 2004; Simpson and Kelly, 2011). For example, the inability to express behaviour may act as a stressor itself, when highly motivated behaviours are thwarted (e.g. Dawkins, 1990; Clubb and Mason, 2003; Mason and Latham, 2004; Vickery and Mason, 2004). In addition, restrictive environment of laboratory cages may limit the ability of the animals to predict and control the environment, and could thus be stressful (Wiepkema and Koolhaas, 1993; Sambrook and Buchanan-Smith, 1997; Bassett and Buchanan-Smith, 2007).

Therefore, recognizing the limitations of the restrictive conditions of laboratory housing environments has led to the emergence of attempts to improve these conditions. Environmental enrichment may be an effective tool of improving the artificial housing conditions of laboratory animals. A consensus definition of environmental enrichment does not exist in the literature therefore, definitions of environmental enrichment vary widely. Environmental enrichment has been defined as any measure which promotes expression of natural, species-specific behaviours and a decrease in, if not disappearance of, abnormal behaviours (Brinkman, 1996). In accordance with that, Chamove (1989a) and Van de Weerd and Baumans (1995) defined environmental enrichment as an “alteration to the living environment of captive animals in order to provide opportunities to express more of their natural behavioural repertoire”. It has also been defined as the “modifications of the environment resulting in an improvement in the biological functioning of the captive animals” (Newberry, 1995). Another definition of environmental enrichment was suggested by

Rosenzweig and Bennett (1996) in which the term was used to refer to the exposure of laboratory animals to physical and/or social stimulation that is greater than they would receive under standard housing conditions. De Azevedo et al., (2007) thought of the term environmental enrichment as the addition of stimuli or provision of choice that results in the improvement of animal well-being. In a similar manner, the term environmental enrichment was used to describe a wide variety of husbandry activities that provide appropriate environmental stimulation to improve the welfare of captive animal (Swaisgood and Shepherdson, 2005). However, despite the variation in the definitions of environmental enrichment, the main goal of environmental enrichment is basically to improve the welfare of animals housed in confinement (Shepherdson et al., 1999; Young, 2003).

Animals have been kept in captivity for scientific purposes for almost 200 years (Young, 2003; O'Regan and Kitchner, 2005; Csatádi et al., 2008), however it was only in the middle of the 19th century that public pressure for more naturalistic enclosures for animals kept in captivity lead to the issuing of guidelines and legislation to improve the welfare of all captive animals, encompassing not only zoos, but also farms and research laboratories (Kulpa-Eddy et al., 2005; Baker et al., 2007). The importance of enrichment for laboratory rodents was first formally acknowledged by Donald Hebb in the 1940s who found that rats reared as pets in his house had superior problem-solving and learning abilities compared to those housed in standard laboratory conditions (Hebb, 1947).

Research has shown a wide range of positive effects to enriching laboratory rats' environment including behavioural (Kaliste et al., 2006; Zaias et al., 2008; Abou-Ismail et al., 2010; Lidfors et al., 2014), physiological (Eckert et al., 2010; Azar et al., 2012; Sharp et al., 2014), psychological (Chamove, 1989b; Patterson-Kane et al., 1999), developmental

(Mirmiran et al., 1982; Kempermann et al., 1997; van Praag et al., 2000), therapeutic and recovery from neural deficits (Jenks et al., 2013; Darwish et al., 2014; Greifzu et al., 2014) cognitive ability and memory, (Harris et al., 2008; Lyst et al., 2012; Jenks et al., 2013) and physical growth and functions and development of the brain (Nithianantharajah and Hannan, 2006; de Carvalho et al., 2010; Eckert et al., 2010; Skillings et al., 2014). Moreover, it has been shown that animals exposed to human and environmental enrichment (novel objects) early in life were easier to handle, less sensitive to novel situations they experience in later life, easier to catch (Csatádi et al., 2005; Moons et al., 2004), and displayed some improved parameters of wellbeing and various stress responses (Belz et al., 2003; Cloutier et al., 2013; Del Arco et al., 2007; Klein et al., 1994). These positive effects of enrichment have been shown to emerge without increasing variability between individuals or disturbing the standardization of the data (Marashi et al., 2004; Würbel, 2007), and this is of a particular importance if the number of animals used for research purposes is to be reduced.

Experimental work on environmental enrichment answered many important questions such as: What is enrichment intended to accomplish (Leach et al., 2000; Varty et al., 2000; Larsson et al., 2002; Toth et al., 2011)? Are the effects of enrichment due to the presence of the enrichments items in the cage or due to rats interacting with the items in their environment (Abou-Ismail et al., 2010)? Are the effects of enrichment due to the presence of multiple items in the cages or a particular item in the rats' environment? (Abou-Ismail, 2011)?

Though much research has been done in the area of environmental enrichment, an important question that remains unanswered is whether the beneficial effects of environmental enrichment arise due to the novelty of items (regular presentation and change of enrichment items over time) or due to the complexity of cages (presence of different permanent items in the cage throughout the housing period).

In order to investigate this issue, which may have implications for the most effective way of providing enrichment to rats, we systematically manipulated the type of enrichment objects provided to rats in their home cages. In one treatment group (Novelty), we placed 5 examples of the same object type (e.g. ladder) into the cage at the start of the study, and then replaced these with 5 examples of a different object (e.g. wooden block) at the start of each following week. This continued for 5 weeks so that the rats experienced 5 different objects, one type at a time, across the study period. In the other treatment group (Complexity), the rats received one example of each of the same 5 types of object as used for the Novelty Group at the start of the study and these were then cleaned and reintroduced into the cage at the start of each of the following 4 weeks. Thus, these animals always had one example of each of the 5 different object types (e.g. ladder, wooden block, etc.) present in their cage throughout the 5-week study period. Cage size and design and social group size were the same for both treatment groups. In this way, we sought to disentangle the relative influence of novelty and complexity of enrichment provision on rat welfare.

2. Methods

2.1. General animal housing

This experiment was carried out using 80 newly weaned male Wistar rats (Al-Alamia, El-Gharbia, Egypt) with an average weight of 41 g at arrival. The experiment was replicated five times, and each treatment was duplicated within each replicate comprising sixteen animals. Young Wistar rats were studied because they represent one of the most common ages and strains used in laboratories. In addition, there is evidence that animals that are exposed to stressful situations during early life may show a long-lasting improvement in their coping mechanisms to challenging situations in adulthood (e.g. Caldji et al., 2000). Males were studied because they are the most commonly used gender used in research (Simpson and Kelly, 2011), partly because female reproductive cycles are assumed to affect behavioural and physiological profiles (e.g. Contreras et al., 2000), which in turn may interfere with experimental findings.

The rats were housed in the same room throughout the study on a 12:12 hour light/dark cycle (lights on 0300–1500) with a continuous dim red light allowing observation in the dark phase. The room was maintained at a constant temperature (20±2 °C). Pelleted food (Rat chow®, Oil and Soap Manufacturing Company, El-Gharbia, Egypt) and tap water were provided ad-libitum and were refreshed daily. Cages contained sawdust (as bedding material) and shredded paper (as nesting material), were checked daily and were cleaned out on a weekly basis six days before behavioural observations were conducted.

The rats were dye-marked (Bigen Oriental Black 59, Hoyo, Nagoya, Japan) to allow individual identification, and these marks were refreshed every two weeks throughout the experimental period, six days before behavioural observations were conducted. Tails were additionally marked to provide another means of identification, should the body marks of the rats be temporarily out of sight.

2.2. Experimental housing conditions

Following acclimatization to the housing conditions and management procedures for 1 wk, rats were arbitrarily assigned to one of the following two housing conditions, in groups of four, for five consecutive weeks:

(1) “Novel housing condition” (NC): Standard polypropylene cages (48.5 cm length × 33 cm width, Al-Alamia, El-Gharbia, Egypt) with elevated cage lids (21 cm height) that were supplied, in a pre-determined order, with five enrichment objects of the same type. These objects were crawl balls (115 mm, with 3 × 58 mm holes, polycarbonate ball, Lillico, UK), aspen wooden blocks (Aspen Wood Block, 34 mm × 70 mm long, local pet store, Pets World, Egypt), shelters (a small enclosed box for rodents, Guinea Pig Huts, 170 x 150 x 110 mm W x L × H, red-tinted polycarbonate, Lillico, UK), ladders (5-step wooden ladder, 220 mm L x 70 mm W, local pet store, Pets World, Egypt) and nylabones (Nylabone, regular size (36g), original flavour, Lillico, UK) (Picture 1). The objects were introduced clean to the cage, and were replaced with other clean objects but of a different type every week over the five-week experimental period. For shelters only, cages were supplied with four items instead of five. This was due to the infeasibility of including five retreats into the cage because of the constraints of cage space. In order to allow the inclusion of four retreats into the cage, the original length of the retreat (205 mm) was reduced to 150 mm. For this housing condition, the type of enrichment items, and the order of their supplementation into the cage changed every week.

(2) “Complex housing condition” (CC): Standard polypropylene cages (48.5 cm length × 33 cm width) with elevated cage lids (21 cm height) that were supplied with 5 enrichment objects of different types (one item of each type). These objects were exactly the same types as those used in the NC group, and were removed, cleaned and re-introduced to the cage after cage cleaning every week, except for malleable objects such as aspen wooden blocks and wooden ladders which were renewed every week. Therefore, the number and type of enrichment items, and the order of their presentation to the animals remained unchanged for this housing condition. The number, type, description and order of supplying enrichment items to the cages are presented in Table 1.

Table One

2.3. Data collection

2.3.1. Behaviour

For all individual rats, behavioural categories were always sampled by the same experienced observer, who entered the experimental room 10 minutes before the scheduled start of the observation to allow the rats to habituate to his presence. Observation was carried out every week in two sessions per day (representing one observation week) for each of the two housing conditions. The first session was carried out during the light phase, starting at 12:30 h and ending at 14:30 h, whereas the second session was conducted during the dark phase of the day; starting at 15:00 h and ending at 17:00 h. Behaviour categories sampled in this experiment are listed in Table 2.

Behaviours of the four rats in each cage were recorded in real time using instantaneous sampling with 10-s intervals between each consecutive focal animal. Each sample interval was dictated by an audio cue via headphones, and the behaviour recorded onto a check sheet. Each session therefore yielded 45 scans per rat. This represented a total of 90 scans per rat per day (observation week), and a total of 450 scans per rat over the entire experimental period. The behaviour of each individual rat was recorded and its position within the cage (underneath food hopper or in-the-open part of the cage) and state (contacting or away from enrichment) was also recorded.

Table Two

2.3.2. Body weight, weight gain and organ weight

Rats were weighed weekly using equilibrated balance. After the data collection was finished, rats were euthanased by an overdose of a barbiturate agent (Thiopental, Egyptian International Pharmaceutical Industries Company, EIPICO, 10th of Ramadan City, Egypt), given by intraperitoneal injection. Immediately after euthanasia the weight (g) of each individual rat was recorded using a digital scale (Oertling, OB033, UK). Each rat was then dissected and the predetermined internal organs (thymus gland, spleen and adrenal glands) were removed and stored on ice in sterile physiological salt solution. These selected internal organs were then trimmed and weighed (mg).

2.4. Statistical analyses

All statistical analyses were carried out using SPSS (version 16.0 for windows). A repeated measures General Linear Model (GLM) was used with week (week 1-5) and session (session 1-2) as within subject factors because behavioural and performance data were collected from the same cages at two different time points every week. Housing condition (complex and novel) was included as a between-subjects factor along with replicate (1-6). Data met requirements of parametric statistics (normality, linearity and homogeneity of variance). The significance value taken was $P<0.05$. The average % of scans (cage average) spent in performing each behaviour was calculated by dividing the total number of scans for each behaviour variable by the total number of scans for each individual rat in each session, and each figure was then multiplied by 100.

To test for the effect of replicate, data for each housing condition was treated separately. For each housing condition the total average % scan performing each behaviour was obtained by summing up the average % scan performing the behaviour in both the light and dark phase for each week. The total average % scan performing each behaviour was then stacked into one column in the statistical model to match the replicates (representing different types of enrichment items). Differences between each observation week in different replicates in average % scan performing behaviour were tested using one-way ANOVA.

The relative weight gain (%) was determined by dividing the value of the absolute weight gain by the value of the body weight in the previous week, and then the figure was multiplied by 100. The organ weights were expressed as a ratio of the body weight (relative weight for each organ). Differences between housing conditions in the final body weight and relative weight of organs were tested using an independent-samples *t*-test. Data are presented as means ± SE.

3. Results

3.1. Behaviour

3.1.1. Main effects

Several behaviour patterns recorded in this study showed an effect of the housing conditions: sleep ($F_{1,37}$= 348.94, P< 0.001); enrichment-directed behaviour ($F_{1,37}$= 64.75, P< 0.001); being in the open part of the cage ($F_{1,37}$= 186.29, P< 0.001) and contacting enrichment ($F_{1,37}$= 553.13, P< 0.001) (Figure 1) were shown more by rats in the CC than those in the NC. Awake non-active ($F_{1,37}$= 87.68, P< 0.001); non-aggressive social interaction ($F_{1,37}$= 11.58, P< 0.001); underneath food hopper ($F_{1,37}$= 181.12, P< 0.001) (Figure 1) and away from enrichment ($F_{1,37}$= 551.02, P< 0.001) were displayed at higher levels by rats in the NC than those in the CC.

3.1.2. Interaction effects

Average % scans spent showing bedding-directed behaviour showed a significant housing condition*observation week effect ($F_{4,37}$= 9.31, P< 0.01), decreasing in the second and third observation week in rats in CC compared to those in NC (Figure 2). Similarly, the average frequency of audible vocalizations showed a significant housing condition*observation week effect ($F_{4,37}$= 1.23, P< 0.05), decreasing in first, second, third and fourth week in CC rats relative to those housed in NC (Figure 3).

Average % scans spent feeding ($F_{4,37}$= 31.03, P< 0.01), drinking ($F_{4,37}$= 3.94, P< 0.05), and performing agonistic behaviour ($F_{4,37}$= 41.85, P< 0.001) (Figure 4) showed a significant housing condition*observation session, decreasing significantly in the dark phase in the CC rats relative to those in NC.

3.2. Body weight, weight gain and organ weight

There was no effect of housing conditions on the body weights of the rats ($F_{1,37}$= 0.67, NS). There was however an effect to the housing conditions on the final weight gain of the rats (t_{38}= -2.59, P<0.05) with rats of the CC gaining more weight than those in the NC (Figure 5).

The independent samples *t*-test demonstrated that housing rats in novel versus complex housing conditions had a significant effect on the average relative thymus gland weight (g) (t_{38}= -2.29, P<0.05) with the rats housed in the CC having heavier thymus than rats housed in the NC (Figure 5). On contrary, there was no effect of housing conditions on the average relative weight of either spleen (t_{38}= -0.86, NS) or adrenal glands (t_{38}= 0.97, NS). Means of some raw behavioural and non-behavioural data collected from the rats in the two housing conditions are shown in Table 3.

3.3. Effects of replicate

For the NC dataset, there was a significant effect of replicate on some behavioural parameters recorded in the study including: sleep ($F_{4,15}$= 4.16, P< 0.05), awake non-active ($F_{4,15}$= 3.30, P< 0.05), feeding ($F_{4,15}$= 3.76, P< 0.05), agonism ($F_{4,15}$= 3.68, P< 0.05), underneath food hopper ($F_{4,15}$= 8.44, P< 0.01), in the open part of the cage ($F_{4,15}$= 8.44, P< 0.01), contact enrichment ($F_{4,15}$= 15.89, P< 0.001) and away from enrichment ($F_{4,15}$= 15.89, P< 0.001). These effects were not found in the CC dataset.

Figure One.

Figure Two.

Figure Three.

Figure Four.

Figure Five.

Table Three.

4. Discussion

The aim of this experiment was to examine whether enriching standard cages of laboratory rats with physical structures produces its effect through the presence of different physical items in the cage at the same time (‘complexity’) or through regularly changing enrichment items whilst only having one type of enrichment in the cage at any one time (‘novelty’). The results show clear differences between rats experiencing complex and novel housing environments in various measures related to welfare. Rats experiencing the complex housing condition demonstrated higher levels of indicators suggestive of improved welfare such as sleep, enrichment-directed behaviour, being in-the-open part of the cage, total weight gain and higher relative weight of thymus gland, and lower levels of indicators suggestive of compromised welfare such as dark phase aggression , non-aggressive social interaction, awake non-active behaviour, away from enrichment, underneath hopper and audible vocalization (in 1st, 2nd, 3rd and 4th week), relative to rats in the novelty treatment group.

Sleep, enrichment-directed behaviour, in-the-open part of the cage have been linked to improved welfare state in laboratory rodents and in rats in particular (Abou-Ismail, 2011; Abou-Ismail et al., 2015). Higher total weight gain and heavier thymus gland weight have also been demonstrated to denote good welfare in laboratory rats (Manser, 1992; Blanchard et al., 1995; Haller et al., 1998; Tsai et al., 2003; Živkovic et al., 2005). Similarly, aggression, non-aggressive social interaction, awake non-active behaviours, away from enrichment, underneath hopper and audible vocalizations have been used to judge the welfare of laboratory rodents with elevated levels being indicative of a generally compromised welfare state (Adams, 1977; Vivian and Miczek, 1993; Marashi et al., 2004; see also Fureix and Meagher 2016). One reason why non-aggressive social interaction may be related to poor welfare is that it may induce aggressive responses in laboratory rodents by increasing the chance that two rats in a cage meet or come in to direct contact with each other. It has been shown that haphazard collision of two running rats or a rear approach and rump or anogenital touching of one unaware rat by another may induce an aggressive response (e.g. Robitaille and Bovet, 1976). Close proximity of animals was also reported to increase aggression in mice (Van Loo et al., 2001).

In rats, the production of audible vocalizations (within the human hearing range) appears to be linked to stressful situations such as attack encounters and defensive behaviours (Blanchard et al., 1986; Kaltwasser, 1990; Vivian and Miczek, 1993), to pain such as electric tail shock (Van Der Poel and Miczek, 1991) and to handling (Adams, 1977), and may therefore reflect a negative welfare state in rodents.

The finding that rats in the novelty condition spent more time eating than those in the complexity condition has parallels with findings that unenriched rats spend more time feeding than those in enriched cages (Denny, 1975; Fiala et al., 1977; Townsend, 1997; Zaias et al., 2008). The more limited set of alternative activities available in the novelty condition may have promoted increased eating behaviour, as may have the larger amount of time spent under the food hopper (which was positively correlated with the total time spent feeding). In humans, some people overeat in response to stress (stress-induced (emotional) eating; Greeno and Wing, 1994), and negative emotions impair cognitive control in restrained eaters (individuals who control their food intake to maintain or reduce their current weight) leading to an increase in food intake (Wallis and Hetherington, 2004). Overeating may also occur in order to shift attention from aversive stimuli and decrease negative affect as a means of emotion regulation (Desmet and Schifferstein, 2008). Similar effects may have been occurring in rats in novel housing conditions if they were more stressed than those in the complexity condition (e.g. as evidenced by greater levels of disrupted sleep, agonistic behaviour and audible vocalizations).

The question that arises now is why animals experiencing the complex housing environment displayed indicators of higher levels of welfare relative to those housed in novel housing conditions? One reason could be that the permanent presence of large space-occupying objects provided an additional amount of space within the cage (e.g. on the objects) and the potential for extra shelter (Black et al., 1989; Patterson-Kane et al., 1999; Moncek et al., 2004). For example, rats in the complexity condition may have used the shelter to avoid white light. Unavoidable white light has been shown to be stressful for nocturnal animals, and in previous studies laboratory rats exposed to it displayed lower levels of both rapid eye movement sleep and short wave sleep (Fishman and Roffwarg, 1972; Borbély, 1978; Alföldi et al., 1990; Tobler et al., 1994). Increased behaviourally recorded sleep for laboratory rats housed in cages enriched with physical structures have also been reported (Mirmiran et al., 1982; Van Gool and Mirmiran 1986; Orok-Edem and Key, 1994; Salin-Pascual et al., 2002). Rats in the novel housing conditions only had access to large enrichment objects such as shelters during the weeks when these were made available, and not during those weeks when they were provided with nylabones, aspen blocks and ladders.

Another reason for improved welfare in rats housed in the complex environments could be that large enrichment objects acted to compartmentalize the cage. Cage compartmentalization has been suggested to provide laboratory rats with refuges from conspecifics' aggressive attacks (Abou-Ismail, 2011), and in the current study, agonistic behaviour was lower in the complex housing condition compared to the novelty condition in which large objects were only available during a limited number of weeks.

Rats experiencing the complex environment may have had improved welfare because the diversity of the enrichment objects provided at any one time was more representative of a varied natural environment, allowing the animals to satisfy any "behavioural needs" (Broom and Johnson, 1993; Jensen and Toates, 1993) to carry out certain behaviours in particular contexts and at particular times. For example, shelters may have allowed rats to choose to rest or sleep at any time of the day, whilst objects such as the wooden blocks and nylabones would allow feeding related behaviour, and ladders and crawl balls would allow climbing. Similarly, objects such as the shelter and crawl balls may have enhanced opportunities for thigmotaxis (wall-hugging) which rats are highly motivated to show (Anzaldo et al., 1994, 1995). They may also have decreased general neophobia (Cowan, 1977; Inglis et al., 1996; Meehan, 1984; Moors et al., 1992), as found by Ennaceur et al. (2006, 2009). Rats in the novelty condition were more constrained in the activities they could choose to perform by the nature of the objects present during any one week.

The ability to exert *control* over the environment by, for example, avoiding the disruptive effect of white light, escaping conspecific attacks, and allowing greater expression of thigmotactic behaviour, may itself have been beneficial for rats housed in the complex environment. Lack of control and predictability can adversely affect animal welfare (Bassett and Buchanan-Smith 2007; Chappell et al., 1986; Dawkins, 1990; Hutton and Wood-Gush, 1983; Joffe et al., 1973; Wiepkema, 1987; Wiepkema and Koolhaas, 1993).

The effect of replicate detected for the novelty but not the complexity condition may be explained by the fact that each replicate in the novelty condition experienced a different order of enrichment objects. For example, if the withdrawal of a valued objects (e.g. shelters) had negative consequences (cf. withdrawal of straw from pigs (Statham et al. 2011)) then these may have been more pronounced in those replicates that received the objects earlier in the 5-week study period. The complexity condition did not experience addition and withdrawal of different types of object, and also showed no effect of replicate.

The above findings together indicate that attention should be paid to the type of enrichment objects provided to rats, and that providing a range of objects simultaneously may be more beneficial than regularly replacing one type of object with a novel one. Replacing enrichment items every week has similarities with housing rats in cages containing different enrichment items which has been shown to produce different responses and to vary in its effects on welfare (Abou-Ismail, 2011; Dallaire et al., 2012; Campbell et al., 2013; Sharp et al., 2014).

Our findings support those of others that the behavioural response of animals towards enrichment is influenced by the the type of the objects provided (rats: Kemppinen et al., 2009; Swetter et al., 2011; Toth, 2015; mice: Bennett et al., 2006; Lockworth et al., 2015). For example, items such as retreats and crawl balls may simulate tunnels and burrows and provide a sense of safety (e.g. from white light and conspecifics: Townsend, 1997; Manser et al., 1998; Eskola et al., 1999), enhance the options for expressing thigmotactic behaviour, and also increase willingness to utilize the open part of the cage (Anzaldo et al., 1994, 1995; Lawlor, 2002; Abou-Ismail et al., 2010). Consistent with this, laboratory rats show a strong preference for objects that provide enclosed spaces within the cage such as retreats, nest boxes and polypropylene standard mouse (M2 type) cages (Bradshaw and Poling, 1991; Chmiel and Noonan, 1996; Townsend, 1997; Manser et al., 1998; Patterson-Kane et al., 2001). The types of enrichment item and the way in which they are provided should thus be carefully considered when designing an environmental enrichment protocol for captive animals such as laboratory rodents.

Enrichment programs can be considered efficient if they improve the welfare of animals by, for example, maximizing the utilization of the environment, increasing the animal's ability to cope with challenges, enhancing performance of species-specific behaviour, and diminishing undesirable behaviours (Kitchen and Martin, 1996, Leach et al., 2000; Van de Weerd and Baumans, 1995; Chamove and Moodie, 1990; Young, 2003; Würbel 2001). It has also been shown that they can be beneficial for welfare without increasing variation in data and, at the same time, may increase the external validity of studies (Richter et al. 2009; Van de Weerd et al., 2002; Würbel and Garner, 2007; Würbel, 2007). Therefore, enrichment may not only improve the welfare of laboratory rodents, but also the overall validity of research, hence resulting in a reduction in the number of animals used.

Further studies are needed to investigate the effects of enrichment novelty versus complexity on other measures of welfare in group-housed laboratory rats such as physiological (e.g. plasma levels of corticosterone, heart rate and blood pressure), psychological (e.g. elevated plus maze, open field and preference test), pathological (histopathology of internal organs) and immunological measures (e.g. total level of the immunoglobulins in the blood or other body fluids and invitro rate of proliferation response of T-lymphocytes to stimulation with mitogens).

5. Conclusion

Housing rats in complex environments (enriched with diverse objects) relative to housing them in novel environments (enriched with frequently changing objects) intensified the expression of various behaviours suggestive of improved welfare such as sleep, enrichment-directed behaviour, contacting enrichment, and being in the open part of the cage, and increased weight gain and relative thymus gland weight. It also diminished the expression of behaviours indicative of reduced welfare such as aggression, awake-non active, bedding-directed behaviour, being underneath the food hopper and audible vocalizations. Enriching laboratory cages with permanent multiple physical structures may have the potential to increase the frequency and diversity of positive natural behaviours, maximize the utilization of the environment, and increase their opportunities for control over the environment, changes that can ultimately result in an improvement in their welfare.

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Figure 1: Means ± SE 'average % scan sleep, awake non-active, underneath hopper, in-the-cage, enrichment-directed, contact enrichment and non-aggressive social interaction' by the rats in the two housing conditions. ** P< 0.01 *** P< 0.001

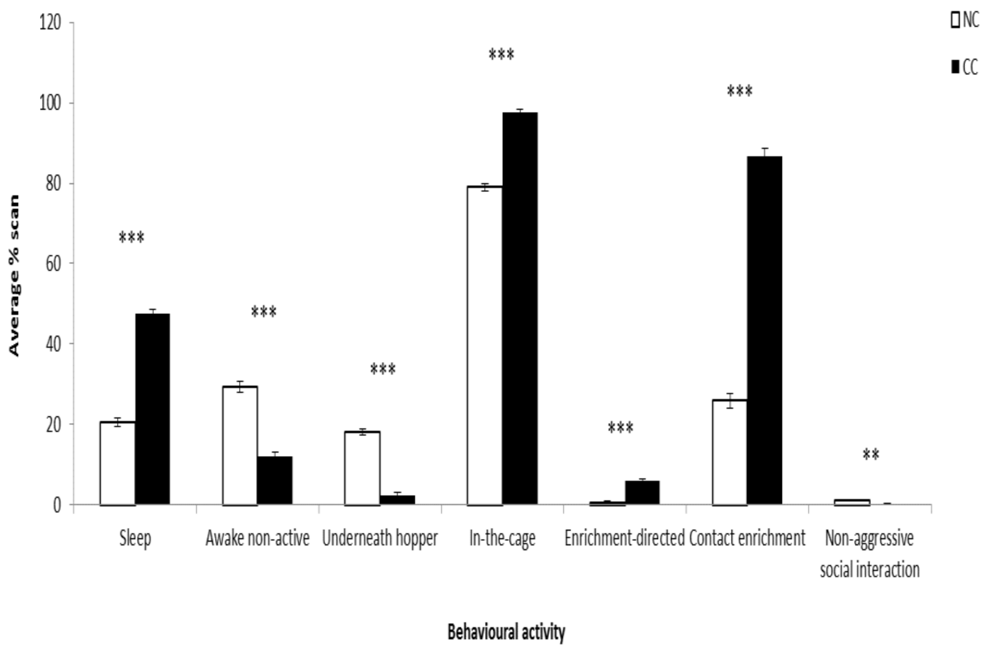


Figure 2: Means ± SE 'average % scan bedding-directed behaviour' by the rats in the two housing conditions. NS non-significant * P< 0.05 ** P< 0.01

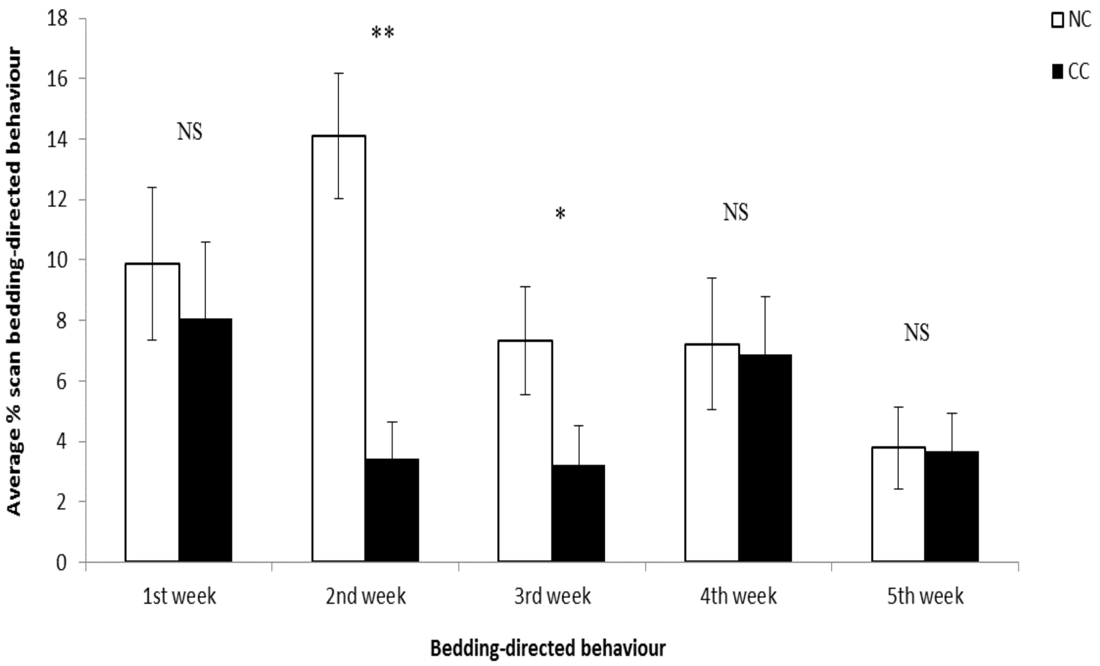


Figure 3: Means ± SE 'average frequency of audible vocalizations' by the rats in the two housing conditions. NS non-significant * P< 0.05

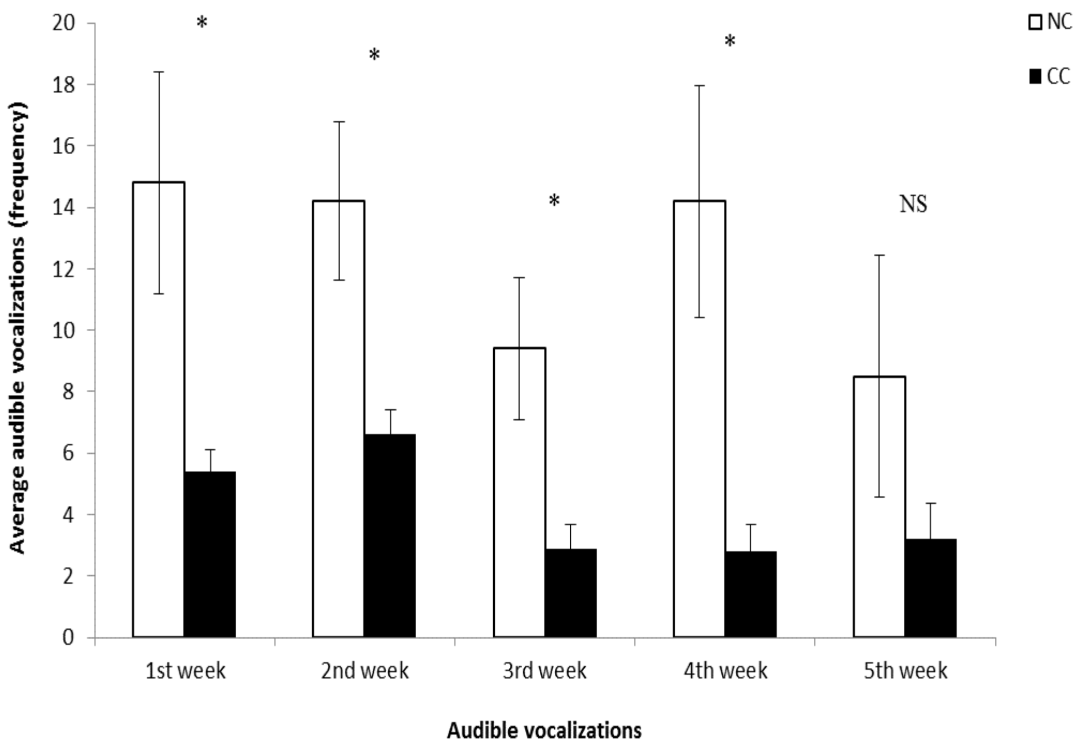


Figure 4: Means ± SE 'average % scan feeding, drinking and agonism' by the rats in the two housing conditions. NS non-significant * P< 0.05 ** P< 0.01 *** P< 0.001

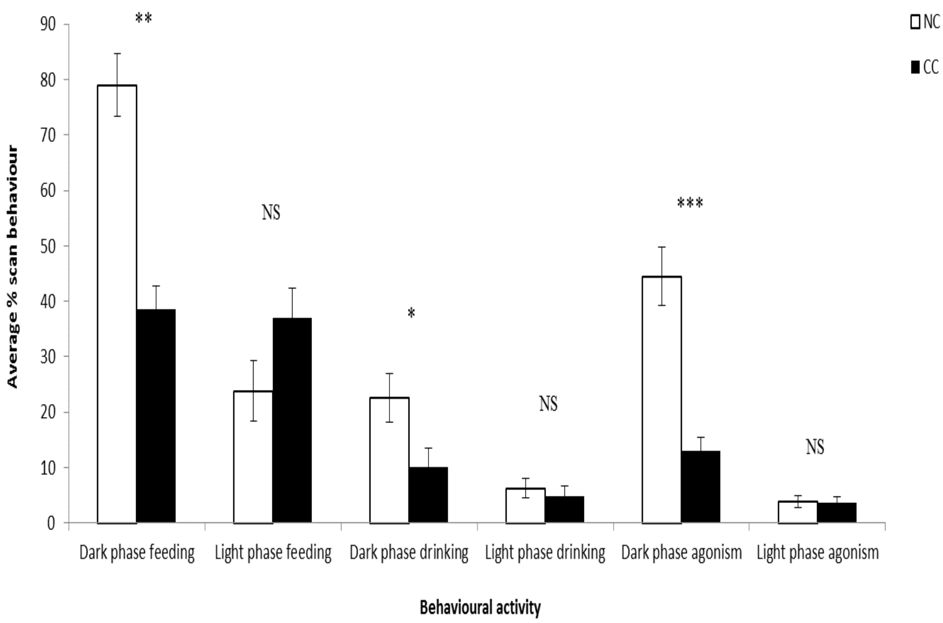
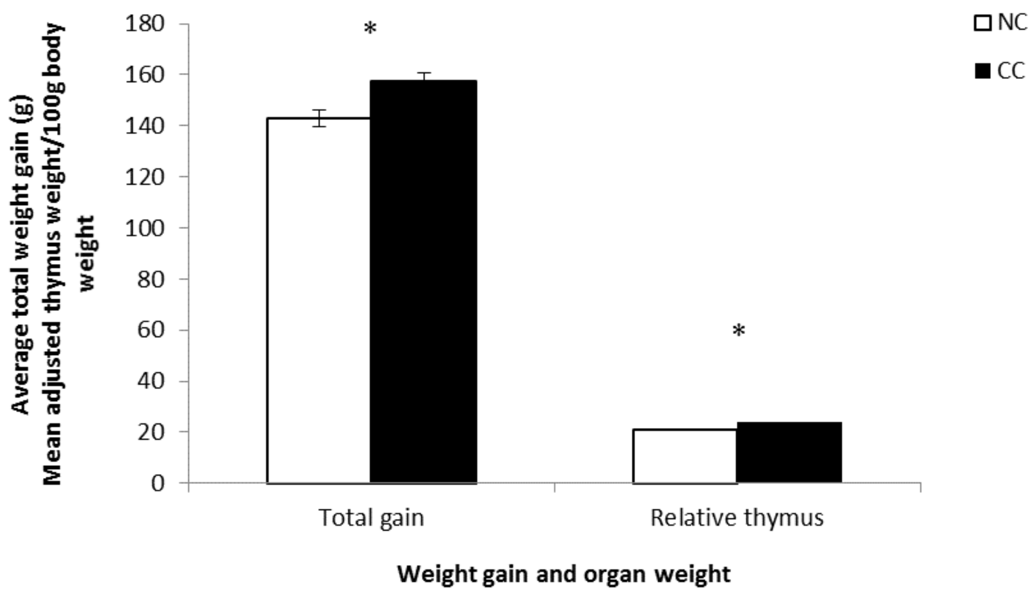


Figure 5: Means ± SE 'average total weight gain (g) and relative thymus gland weight (g)' by the rats in the two housing conditions. * P< 0.05



Picture 1: Type of cage and enrichment items used.

Table one. Number, type, description and order of supplying enrichment objects to the cages.

	Replicate 1		**Replicate 2**		**Replicate 3**		**Replicate 4**		**Replicate 5**	
	Housing condition		**Housing condition**		**Housing condition**		**Housing condition**		**Housing condition**	
	Novel (2 cages)	**Complex (2 cages)**	**Novel (2 cages)**	**Complex (2 cages)**	**Novel (2 cages)**	**Complex (2 cages)**	**Novel (2 cages)**	**Complex (2 cages)**	**Novel (2 cages)**	**Complex (2 cages)**
Week 1	5 Crawl balls	5 items (one item of each type). A crawl ball, ladder, aspen wooden block, nylabone and a shelter	5 Ladders	5 items (one item of each type). A crawl ball, ladder, aspen wooden block, nylabone and a shelter	5 Aspen wooden blocks	5 items (one item of each type). A crawl ball, ladder, aspen wooden block, nylabone and a shelter	5 Nylabones	5 items (one item of each type). A crawl ball, ladder, aspen wooden block, nylabone and a shelter	4 Shelters	5 items (one item of each type). A crawl ball, ladder, aspen wooden block, nylabone and a shelter
Week 2	5 Ladders		5 Aspen wooden blocks		5 Nylabones		4 Shelters		5 Crawl balls	
Week 3	5 Aspen wooden blocks		5 Nylabones		4 Shelters		5 Crawl balls		5 Ladders	
Week 4	5 Nylabones		4 Shelters		5 Crawl balls		5 Ladders		5 Aspen wooden blocks	
Week 5	4 Shelters		5 Crawl balls		5 Ladders		5 Aspen wooden blocks		5 Nylabones	

Table two. Ethogram for behavioural elements recorded.

Category	Behavioural component	Description
A- General activities	1- Feeding	Eating food or faecal pellet
	2- Drinking	Drinking water from waterspouts
	3- Non-intake maintenance	Self-grooming, sneezing and pandiculation (stretching and yawning)
	4- Movement activities	Movement, running and jumping
	5- Exploratory behaviour	Sniffing cage wall, cage roof (top and sides) and sniffing air outside the cage
	6- Bedding-directed behaviour	Sniffing bedding, bedding manipulation, burrowing, digging and eating floor substrate
	7- Non-aggressive social interaction	Social investigation (sniffing) and allogrooming (given and received) any region of the body of cage mate
B- Sleep	1- Sleep	Lying unalert with both eyes closed-apparently asleep
C- Agonism	1- Agonism	Aggression, defence, dominant over (the winner rat positioned over the loser with its forepaws placed on the latter, regardless of who initiated or received the aggressive act), on-back-posture (the loser rat lies on its back, fully exposing its ventral surface to the winner, regardless of who initiated or received the aggressive act), and aggressive grooming (given and received)

D- Enrichment-directed behaviour	1- Enrichment-directed	Sniffing, chewing, climbing, and manipulating the enrichment objects.
E- Other behaviour	1- Awake non-active	Stationary- alert (eyes open) but with no directed attention while lying or standing
	2- Audible vocalizations	The emittence of audible vocalizations by any rat of the group; for this behaviour only the identity of the animal that produced the vocalization was unknown
	3- Out of sight	Behaviour of the focal rat cannot be observed
F- Position in the cage:	1- Underneath food hopper	When the whole body of the rat, excluding its tail, is entirely underneath the food hopper or waterspouts at the moment of the scan
	2- In-the-open part of the cage	When the whole body of the rat, including its tail, is entirely in the open part of the cage
G- State of the animal:	1- Contacting enrichment	When the body of the rat, being active or not, excluding its tail, is in a direct physical contact with the enrichment object
	2- Away from enrichment	When the body of the rat, being active or not, excluding its tail, is not in a direct physical contact with the enrichment object

Table three. Mean % scan behaviour, mean frequency audible vocalizations, mean relative organ weight (g) and mean body weight (g) by the rats in the two housing conditions ± SE. * Difference between means in the same row is significant.

Measure	**“Novel housing condition”**	**“Complex housing condition”**
Sleep	20.51 ± 1.03	47.58* ± 1.03
Move	1.92 ± 0.39	2.06 ± 0.39
Awake non-active	29.48* ± 1.32	11.94 ± 1.32
Agonism	5.83* ± 0.41	1.89 ± 0.41
Feeding	11.10* ± 0.64	8.56 ± 0.64
Drinking	2.99* ± 0.47	1.40 ± 0.47
Non-intake maintenance	16.90 ± 1.15	19.67 ± 1.15
Exploration	2.83 ± 0.43	2.24 ± 0.43
Bedding-directed behaviour	4.20 ± 0.44	2.56 ± 0.44
Non-aggressive social interaction	1.07* ± 0.17	0.26 ± 0.17
Audible vocalizations	6.10* ± 0.40	1.92 ± 0.40
Enrichment-directed behaviour	0.61 ± 0.51	6.11* ± 0.51

Underneath hopper	18.11* ± 0.83	2.36 ± 0.83
In-the-open part of the cage	78.95 ± 0.82	97.68* ± 0.82
Contacting enrichment	26.00 ± 1.83	86.81* ± 1.83
Away from enrichment	74.15* ± 1.83	13.29 ± 1.83
Relative thymus weight	0.210 ± 0.012	0.238* ± 0.004
Relative spleen weight	0.501 ± 0.028	0.632 ± 0.149
Relative adrenal weight	0.0105 ± 0.0003	0.0104 ± 0.0004
Final body weight	296.80 ± 7.73	316.4 ± 4.72
Total weight gain	142.90 ± 3.43	157.57* ± 3.12